

The in vitro respiratory and acid–base properties of blood and tissue from the Kemp's ridley sea turtle, *Lepidochelys kemp*

ERICH K. STABENAU¹

National Marine Fisheries Service, 4700 Avenue U, Galveston, TX 77551, U.S.A.

and

Departments of Physiology and Biophysics and of Internal Medicine, University of Texas, Galveston, TX 77550, U.S.A.

AND

THOMAS A. HEMING

Departments of Physiology and Biophysics and of Internal Medicine, University of Texas, Galveston, TX 77550, U.S.A.

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We determined the in vitro respiratory and acid–base properties of blood and tissue from Kemp's ridley sea turtles (*Lepidochelys kemp*). Blood O₂ dissociation curves of ridley turtles were sigmoid, with a P_{50} of 31.2 ± 0.3 (mean \pm SD) torr at 25°C and pH 7.51. Increments in temperature or PCO_2 were associated with a shift of the O₂ dissociation curves to the right and, hence, a reduction in hemoglobin–O₂ binding affinity. The apparent heat of oxygenation, which is a measure of the temperature sensitivity of hemoglobin–O₂ affinity, was -10.5 kcal/mol O₂. The degree of cooperativity of O₂ for hemoglobin binding sites, as measured by the Hill coefficient, increased at higher temperatures (20–30°C at a PCO_2 of 37 torr), but was unaffected by changes in PCO_2 (37–52 torr at 25°C). The CO₂–Bohr effect was -0.34 torr/pH unit. The CO₂ capacitance coefficient of whole blood and plasma declined as a function of increased PCO_2 (22°C). Non-bicarbonate buffer capacities (22°C) were 19.7, 18.5, and 6.4 slykes for whole blood, true plasma, and separated plasma, respectively. The skeletal muscle myoglobin content was 3.1 ± 0.84 mg \cdot g⁻¹ of tissue. The respiratory and acid–base properties of blood and tissue from Kemp's ridley sea turtles are consistent with those of species that utilize lung O₂ stores during long-term aerobic dives. The enhanced hemoglobin–O₂ temperature sensitivity exhibited by the ridley turtle could be a physiological adaptation for life in coastal environments that typically undergo substantial fluctuations in temperature.

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Nous avons étudié in vitro les propriétés respiratoires et l'équilibre acide–base du sang et des tissus chez la tortue marine *Lepidochelys kemp*. Les courbes de dissociation de l'oxygène du sang sont sigmoïdes avec une pression P_{50} égale à $31,2 \pm 0,3$ (moyenne \pm écart type) torr à 25°C, à un pH de 7,51. L'augmentation de la température ou de la pression PCO_2 entraîne un déplacement des courbes de dissociation de l'oxygène vers la droite et, par conséquent, une diminution de l'affinité hémoglobine–O₂. La chaleur apparente dégagée par l'oxygénation, qui constitue une mesure de la sensibilité thermique de l'affinité hémoglobine–O₂, a été évaluée à $-10,5$ kcal/mol O₂. Le degré d'affinité de l'oxygène aux sites de liaison de l'hémoglobine, tel que mesuré par le coefficient de Hill, augmente aux températures plus élevées (20–30°C à une pression PCO_2 de 37 torr), mais n'est pas affecté par des variations de PCO_2 (37–52 torr à 25°C). L'effet CO₂–Bohr a été évalué à $-0,34$ torr/unité de pH. Le coefficient de capacitance du CO₂ du sang entier et du plasma diminue à mesure qu'augmente la pression PCO_2 (22°C). La capacité tampon (sans bicarbonate) à 22°C a été estimée à 19,7, slykes dans le cas du sang entier, à 18,5 slykes dans le cas du plasma intégral et à 6,4 slykes dans le cas du plasma séparé. Le contenu en myoglobine des muscles squelettiques était de $3,1 \pm 0,84$ mg \cdot g⁻¹ de tissu. Les propriétés respiratoires et l'équilibre acide–base du sang et des tissus de cette tortue correspondent à ceux d'autres espèces qui utilisent des réserves pulmonaires d'oxygène au cours des longues plongées aérobies. La sensibilité accrue de l'oxygène de l'hémoglobine à la température chez cette tortue pourrait bien être une adaptation physiologique à la vie dans des milieux côtiers qui subissent fréquemment des fluctuations substantielles de température.

[Traduit par la Rédaction]

Introduction

Sea turtles possess hemoglobins that are structurally and kinetically adapted to release O₂ to tissues at the expense of efficient O₂ loading and storage (Friedman et al. 1985). To compensate for the inefficient hemoglobin–O₂ storage properties, some sea turtles exhibit elevations in erythrocyte hemoglobin content, hematocrit, and tissue myoglobin content, which serve to increase blood and tissue O₂ stores. For example, leatherback sea turtles (*Dermochelys coriacea*) have high blood O₂

carrying capacities and tissue myoglobin contents, which increase blood and tissue O₂ stores and permit dives to depths exceeding 1000 m (Lutcavage et al. 1990). Loggerhead (*Caretta caretta*) and green (*Chelonia mydas*) sea turtles have lower tissue myoglobin contents and blood O₂ carrying capacities, and typically dive to depths of less than 300 m (Berkson 1967; Sakamoto et al. 1990).

Ridley sea turtles, *Lepidochelys kemp* and *L. olivacea*, are the smallest sea turtle species. Adult turtles weigh up to 45 kg and have maximum carapace lengths of ~70 cm. Ridley sea turtles are generally found in shallow-water coastal environments that may undergo substantial seasonal fluctuations in temperature. *Lepidochelys kemp* possesses comparable

¹Author to whom correspondence should be sent at the following address: Department of Physiology, School of Medicine, Brody Building, East Carolina University, Greenville, NC 27858-4354, U.S.A.

hematocrit (Davis 1991; Stabenau et al. 1991), blood volume (Thorson 1968), and hemoglobin content (Davis 1991) to loggerhead and green sea turtles, species that utilize lung O_2 stores during prolonged aerobic dives. Nevertheless, Landis (1965; positive species identification in Kooyman 1988) directly observed a *L. olivacea* feeding at 300 m, well below the expected depth at which lung collapse occurs (i.e., ~120 m; Berkson 1967). No information is available on the respiratory properties of blood (e.g., hemoglobin- O_2 dissociation curves, Bohr effect, blood buffer capacity) and tissue from ridley sea turtles. This study determined the in vitro respiratory and acid-base properties of blood and tissue from Kemp's ridley sea turtles in order to explore the possibility that this shallow-water coastal sea turtle species possesses alternative O_2 storage properties to those found in other sea turtles.

Materials and methods

Venous blood samples were collected into heparinized syringes from the dorsal cervical sinus of 1- to 2-year-old *L. kempi* that were captive-reared at the National Marine Fisheries Service (NMFS), Galveston Laboratory, Texas. The blood samples were stored at 4°C pending in vitro analyses. Preliminary experiments indicated that storage of blood samples for 0, 1, 4, and 24 h did not result in significant changes in blood pH, PCO_2 , PO_2 , [lactic acid], and [ATP] (Student's *t* tests, $P > 0.05$).

Oxygen dissociation curves were constructed using a mixing technique, following the methods outlined by Lutcavage et al. (1990). Briefly, samples of fully oxygenated ($PO_2 = 134$ torr) and deoxygenated whole blood ($PO_2 = 0$ torr) were equilibrated in tonometers at a given PCO_2 (37–52 torr). Aliquots of the two blood pools were mixed anaerobically in a 250- μ L gastight syringe, and the PO_2 and pH of the mixture were measured in duplicate (PO_2 with electrode type E5046 and pH with electrode type G297/G2, Radiometer, Copenhagen, Denmark). Percent O_2 saturation was calculated from the known proportions of oxygenated and deoxygenated blood in the mixture. The effects of temperature and pH on oxygen dissociation curves were examined by equilibrating blood samples over the temperature range 20–30°C and the PCO_2 range 37–52 torr. Individual O_2 dissociation curves were fit with a sigmoid model to the experimental data to obtain P_{50} values (Fig. P Software Corporation, Durham, North Carolina). It is noteworthy that PO_2 of the equilibrated blood samples was not influenced by changes in the concentration of allosteric factors (e.g., ATP) following storage of blood samples at 4°C (see above).

The relationship between in vitro blood pH and temperature was determined using whole-blood samples contained in gastight syringes in a thermostatted water bath. Temperature was increased at 2°C intervals over the range 20–30°C, and whole-blood pH was measured in duplicate after 15 min of equilibration at each temperature.

CO_2 combining curves (total CO_2 content versus PCO_2) and non-bicarbonate buffer curves (total CO_2 content versus pH) were constructed for separated plasma, true plasma, and whole blood at 22°C. Samples (2 mL) of whole blood and separated plasma were exposed individually to 1.5% CO_2 (balance N_2) in tonometers. Total CO_2 content (CCO_2) and pH were measured in duplicate after 15–30 min equilibration. CCO_2 was measured in a CO_2 electrode chamber, as described by Cameron (1971) (CO_2 electrode type E5036, Radiometer). The procedure was repeated using fresh samples of whole blood and plasma at 3.2, 5.1, and 7.8% CO_2 . True plasma was obtained at each CO_2 gas pressure by centrifugation of the equilibrated whole-blood samples and immediate anaerobic removal of plasma. CO_2 combining curves were fit to the experimental data using a log-linear model (Weinstein et al. 1986),

$$[1] \quad CCO_2 = a + b(\log PCO_2)$$

The CO_2 capacitance coefficient (β_{CO_2} , the instantaneous rate of change in CCO_2 per torr PCO_2) was calculated from the slope (first derivative) of the CO_2 combining curve. Blood and plasma PCO_2 was calculated from the known fractional CO_2 concentration, corrected for temperature, barometric pressure, and water vapor pressure. $[HCO_3^-]$ was calculated from a rearrangement of the Henderson-Hasselbalch equation using values for α_{CO_2} and pK_a of the plasma of the ridley turtle predicted from the following equations (Stabenau and Heming 1993):

$$[2] \quad \alpha_{CO_2} = 9.174 \times 10^{-2} - 3.269 \times 10^{-3} T + 6.364 \times 10^{-5} T^2 - 5.378 \times 10^{-7} T^3$$

$$[3] \quad pK_a = 6.398 - 1.341 \times 10^{-2} T + 2.282 \times 10^{-4} T^2 - 1.516 \times 10^{-6} T^3 - \log(1.011 + 10^{pH + 0.011T - 10.241} + 10^{pH + 0.001T - 8.889})$$

where T is temperature (°C).

The myoglobin content of the plantaris muscle from four Kemp's ridley turtles was measured using the spectrophotometric method described by Reynafarge (1963).

Results

Oxygen dissociation curves for *L. kempi* are adequately described as sigmoid ($P \leq 0.05$). P_{50} was 31.2 ± 0.3 (mean \pm SD) torr at 25°C and pH 7.51 (Fig. 1A). Increasing the temperature from 20 to 30°C shifted the O_2 dissociation curves to the right, thereby increasing P_{50} (Fig. 1A, Table 1). A comparable shift in the O_2 dissociation curves accompanied the increase in PCO_2 from 37 to 52 torr (Fig. 1B, Table 1). The temperature sensitivity of hemoglobin- O_2 affinity over the range 20–30°C was measured using the van't Hoff equation:

$$[4] \quad \Delta H = 2.303R(\Delta \log P_{50})/\Delta(1/t)$$

where ΔH is the apparent heat of oxygenation, R is the universal gas constant, and t is absolute temperature (K). ΔH was -10.5 kcal/mol O_2 . The CO_2 -Bohr effect ($\Delta \log P_{50}/\Delta pH$) was -0.34 torr/pH unit. Hill coefficients (n), calculated over the linear O_2 saturation range (15–85%), revealed that increasing the temperature from 20 to 30°C at constant PCO_2 (37 torr) substantially increased the degree of cooperativity of O_2 for hemoglobin binding sites, whereas raising PCO_2 from 37 to 52 torr at constant temperature (25°C) did not alter cooperativity (Table 1).

The in vitro pH of blood varied as a function of temperature (T , °C) (Fig. 2). The $\Delta pH/\Delta T$ of Kemp's ridley sea turtle blood was -0.0175 units/°C, which is comparable to in vivo data for green (-0.014 units/°C; Kraus and Jackson 1980) and loggerhead sea turtles (-0.017 units/°C; Lutz et al. 1989).

CO_2 combining curves for separated plasma, true plasma, and whole blood are illustrated in Fig. 3A. The CO_2 capacitance coefficient, β_{CO_2} , declined logarithmically as a function of increased PCO_2 . There was no difference in the β_{CO_2} values of whole blood and true plasma. However, the β_{CO_2} values of whole blood and true plasma were considerably greater than that of separated plasma at any PCO_2 , which is consistent with an effect of red blood cells on buffer capacity (Fig. 3A). Non-bicarbonate buffer curves are illustrated in Fig. 3B. The non-bicarbonate buffer capacity of *L. kempi* whole blood was 19.7 slykes, which is in general agreement with values reported for whole blood from loggerhead sea turtles (16.4 slykes; Lutz and Bentley 1985) and the freshwater turtle *Phrynops hilarii* (22.6 slykes; Richel

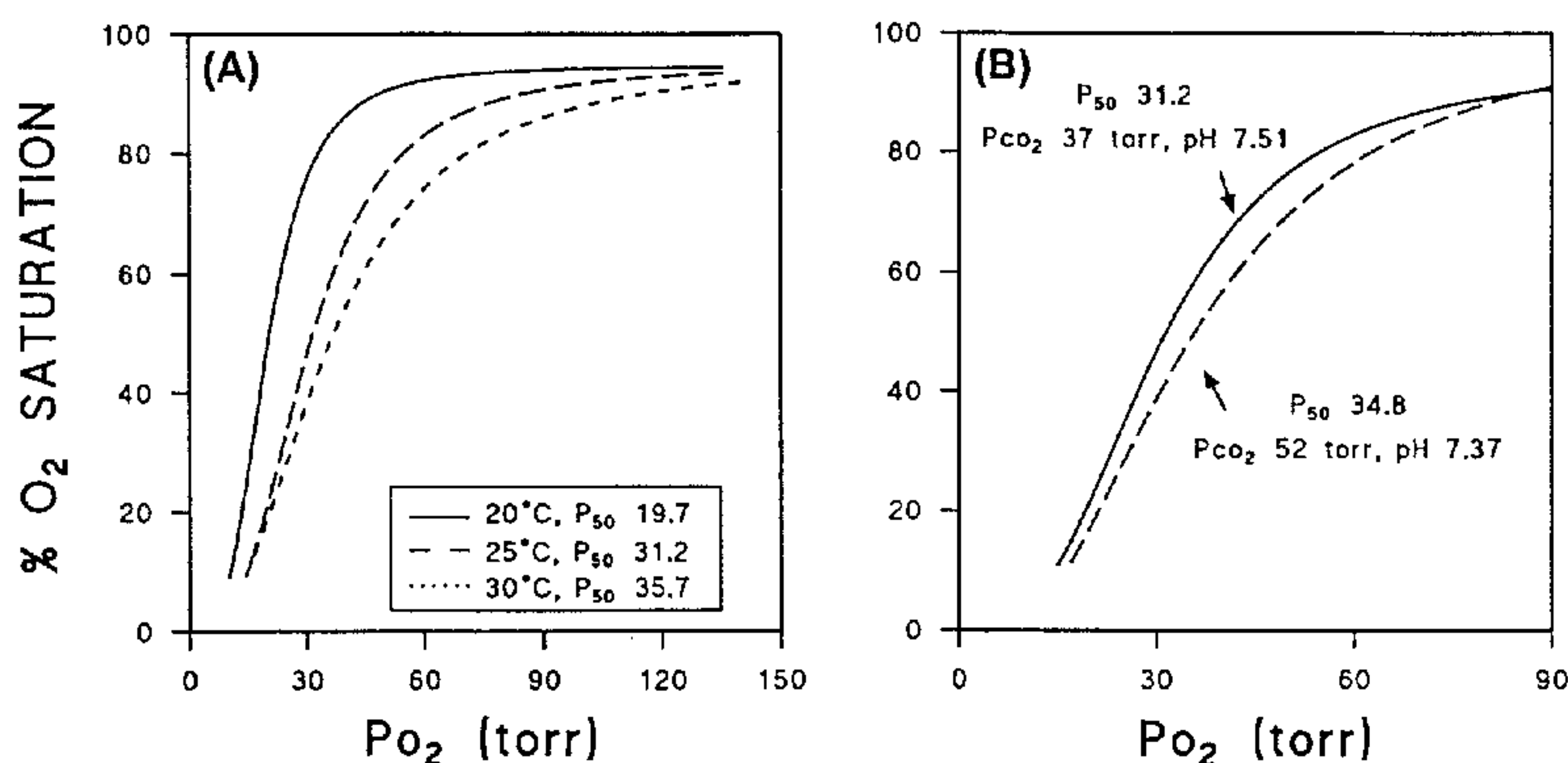


FIG. 1. Effects of temperature (A) and P_{CO_2} (B) on the blood O₂ dissociation curves of Kemp's ridley sea turtle (*Lepidochelys kempi*). Individual curves are best fit relationships to the experimental data (14–26 data points per curve).

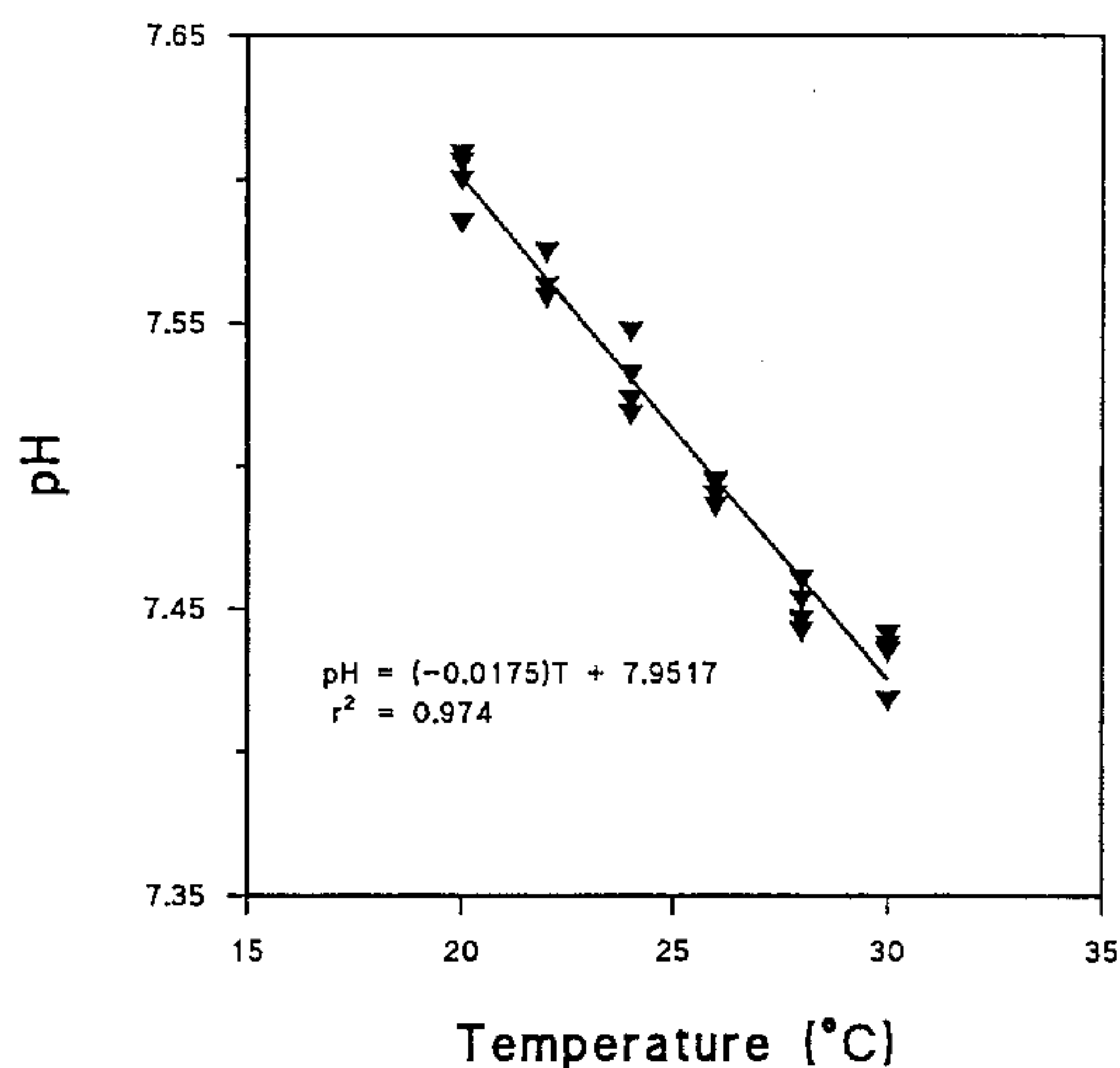


FIG. 2. Relationship between in vitro blood pH and temperature for *L. kempi* over the temperature range 20–30°C.

et al. 1984). In Kemp's ridley turtles, the non-bicarbonate buffer capacity of true plasma was 18.5 slykes and that of separated plasma was 6.4 slykes.

The myoglobin concentration in the plantaris muscle from the Kemp's ridley turtle was 3.1 ± 0.84 (mean \pm SD) $\text{mg} \cdot \text{g}^{-1}$ of tissue ($n = 4$), which is comparable to data reported for the pelvic and pectoral muscles from loggerhead ($2.9 \text{ mg} \cdot \text{g}^{-1}$ of tissue; Lutz and Bentley 1985) and leatherback sea turtles ($4.9 \text{ mg} \cdot \text{g}^{-1}$ of tissue; Lutcavage et al. 1990).

Discussion

Air-breathing vertebrates can be separated into two classes based on their reliance on lung O₂ stores or tissue and blood O₂ stores during long-term dives. Species that rely on tissue and blood O₂ stores tend to have a high tissue myoglobin

content and blood O₂ carrying capacity (Lutcavage et al. 1990). These properties enable animals such as the leatherback sea turtle and various marine mammals to dive to extreme depths. In contrast, sea turtle species that rely on lung O₂ stores during long-term dives tend to have a lower tissue myoglobin content and blood O₂ carrying capacity (Lutz and Bentley 1985). The blood hematocrit, hemoglobin content, O₂ carrying capacity, and tissue myoglobin content of Kemp's ridley sea turtles are substantially lower than those reported for sea turtle and marine mammal species capable of prolonged deep diving. This suggests that *L. kempi* is poorly adapted to use blood and tissue O₂ stores, probably relying instead on lung O₂ stores during long-term aerobic dives.

The proportional pre-dive oxygen stores in the muscle, blood, and lung of Kemp's ridley sea turtles were calculated using equations described by Kooyman et al. (1983). Lung O₂ stores of this species range from 15.5 to 30.0 $\text{mL O}_2 \cdot \text{kg}^{-1}$, with lung volume estimated either as a percentage of body mass (Berkson 1966; Milsom 1975) or from the equation of Tenney and Tenney (1970). Muscle and blood O₂ stores were 1.2 and 9.0 $\text{mL O}_2 \cdot \text{kg}^{-1}$, respectively. Therefore, the proportional O₂ stores are 3.6–4.7% in the muscle, 24.8–35.0% in the blood, and 60.3–75.2% in the lung. These values are comparable to the pre-dive muscle (3.6%), blood (24.8%), and lung O₂ stores (71.6%) estimated for the loggerhead turtle (Lutz and Bentley 1985). Oxygen loading from lung to blood would be limited in sea turtles that utilize lung O₂ stores during diving by lung collapse at depths exceeding ~120 m (Berkson 1967). It is interesting to note that Kemp's ridley sea turtles tracked with satellite telemetry in the Gulf of Mexico and Atlantic Ocean routinely remain in waters less than 100 m in depth (Maurice Renaud, NMFS, personal communication). Thus, the diving capacities of Kemp's ridley sea turtles may be limited by relatively low blood and tissue O₂ storage and reliance on lung O₂ stores.

Oxygen dissociation curves for Kemp's ridley turtles are adequately described as sigmoid, and the degree of cooperativity of O₂ for hemoglobin binding sites (i.e., the Hill coefficient) was similar to values documented for green (Wood et al. 1984), loggerhead (Lapennas and Lutz 1982), and leatherback turtles (Lutcavage et al. 1990). P_{50} for

TABLE 1. Oxygen transport properties of blood from *Lepidochelys kempfi* and other sea turtle species

	Body mass (kg)	Hematocrit (%)	Hb (g/100 mL blood)	O ₂ capacity (mL/100 mL blood)	P ₅₀ (torr)	PCO ₂ ^a	pH	Temp (°C)	Hill coefficient (n)	CO ₂ -Bohr effect	Source
<i>Lepidochelys kempfi</i> (Kemp's ridley)	3-5	29	9.6 ^b	~13 ^c	19.7 31.2 34.8 35.7	37 37 52 37	7.47 7.51 7.37 7.51	20 25 25 30	2.2 2.6 2.5 3.0	-0.34	This study
<i>Caretta caretta</i> (loggerhead)	3-4	29	9.8	~13 ^c	47.0	37	7.45	25	2.7	-0.38	Lapennas and Lutz 1982
<i>Chelonia mydas</i> (green)	3-4 88.2 1.1	30 np np	8.8 8.8 8.8	~12 ^c ~12 ^c ~12 ^c	29.0 18.2 31.3	37 np np	7.45 7.60 7.60	25 25 25	2.4 2.8 3.4	-0.52 -0.59 -0.23	Lapennas and Lutz 1982 Wood et al. 1984 Wood et al. 1984
<i>Dermochelys coriacea</i> (leatherback)	~300 ~300	39 39	15.6 15.6	21 21	31 40	2.2% 4.8%	7.84 7.52	25 25	3.6 2.7	-0.34	Lutcavage et al. 1990

NOTE: np, not provided.

^aTorr, unless noted otherwise.^bFrom Davis (1991).^cComputed as 1.34[Hb].

Kemp's ridley turtles (pH 7.51, PCO₂ 37 torr, 25°C) closely resembled values reported for green turtles, yet was considerably less than that described for loggerhead turtles despite similar body mass, hematocrit, hemoglobin content, and O₂ carrying capacity in the three species (Table 1). The relatively low P₅₀ for Kemp's ridley and green turtles indicates that hemoglobin-O₂ affinity is increased in these species, which promotes O₂ loading over O₂ unloading.

Generally, increments in blood PCO₂ decrease hemoglobin-O₂ affinity (i.e., increase P₅₀) and increase the degree of cooperativity of O₂ for hemoglobin binding sites (i.e., decrease the Hill coefficient), thereby promoting O₂ unloading. The O₂ transport properties of blood from *L. kempfi* were relatively weakly influenced by PCO₂. The hemoglobin-O₂ affinity of Kemp's ridley sea turtle blood decreased slightly with increases in PCO₂, whereas the degree of cooperativity of O₂ was unaffected by the changes in PCO₂. For comparison, Lutcavage et al. (1990) reported a decrease in the Hill coefficient of leatherback turtle blood from 3.6 to 2.7 with an increase in PCO₂ from 2.2 to 4.8%. The relative insensitivity of ridley blood to PCO₂ may represent an adaptation to promote O₂ storage and transport over O₂ unloading. Increases in pulmonary gas PO₂ during lung compression at depth would promote loading of O₂ from lung to blood and may compensate for the insensitivity of ridley blood to PCO₂.

The oxygen transport properties of blood from *L. kempfi* were strongly influenced by temperature. Both P₅₀ and the Hill coefficient increased at higher temperatures, indicating a reduction in hemoglobin-O₂ affinity and an increase in the degree of cooperativity of O₂ for hemoglobin binding sites (Table 1). ΔH, a measure of the temperature sensitivity of hemoglobin-O₂ affinity was -10.5 kcal/mol O₂. This value is similar to those reported for the freshwater turtles *Pseudemys scripta* (-10.5 kcal/mol; Burggren et al. 1977) and *Chrysemys picta* (-12.4 kcal/mol; Maginniss et al. 1983). The large hemoglobin-O₂ temperature sensitivity exhibited by these turtles may be an adaptive mechanism to promote O₂ unloading from blood to tissue during elevations in body temperature (Burggren et al. 1977). Thus, enhanced hemoglobin-O₂ temperature sensitivity could be an adaptation of Kemp's ridley turtles for life in warm waters. The water temperature of coastal environments occupied by *L. kempfi* in the Gulf of Mexico can exceed 30°C in summer.

The in vitro blood pH of *L. kempfi* decreased 0.0175 units/°C with increases in temperature over the range 20-30°C. Such an inverse relationship between blood pH and temperature is well documented for many ectothermic vertebrates (Howell and Rahn 1976; Reeves 1976; Lutz et al. 1989).

The non-bicarbonate buffer capacity of *L. kempfi* whole blood was 19.7 slykes. For comparison, Butler and Jones (1983) reported a blood buffer capacity for other reptile species that are twice the value reported for turtles. Sea turtles also have a low intracellular buffer capacity compared with non-reptilian species (Blomberg and Baldwin 1991). Large pH fluctuations have been observed in blood of sea turtles during diving (Wood et al. 1984) and involuntary submergence (Stabenau et al. 1991). Such changes in blood pH are undoubtedly due, in part, to the low intra- and extra-cellular buffer capacities.

The βCO₂ of *L. kempfi* whole blood, true plasma, and separated plasma decreased as a function of increased PCO₂ (Fig. 3A). Weinstein et al. (1986) described comparable CO₂

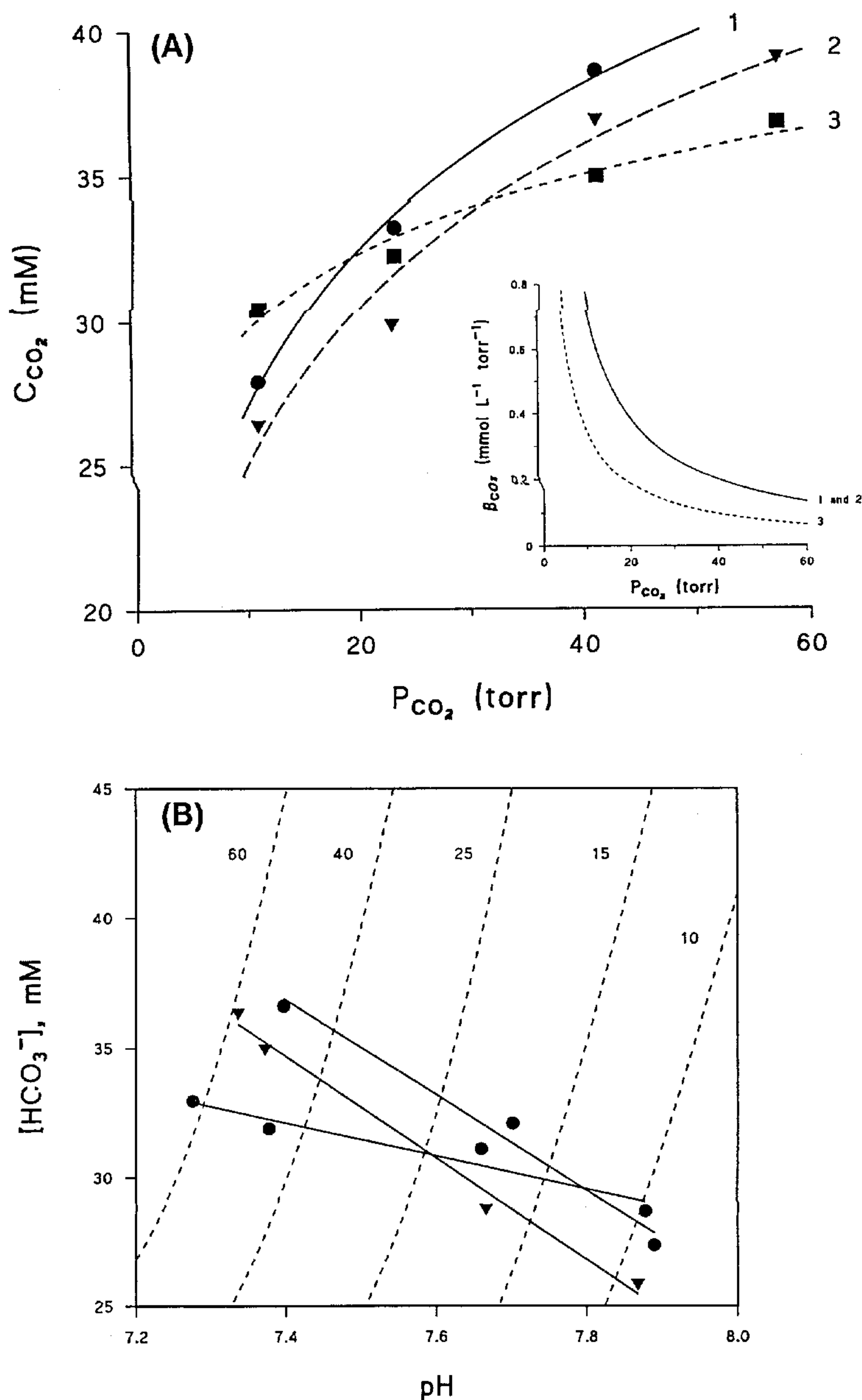


FIG. 3. CO_2 combining curves (A) and non-bicarbonate buffer curves (β) for whole blood (∇), true plasma (\bullet), and separated plasma (\blacksquare) of *L. kempfi*. Data points are means of 3 or 4 replicates. Standard deviations (not shown) were $\leq 10\%$ of the mean values. The inset in A gives the CO_2 capacitance coefficients (β_{CO_2}) of true plasma (1), whole blood (2), and separated plasma (3). The broken lines in B represent P_{CO_2} isopleths from 10 to 60 torr.

combining curves and β_{CO_2} for whole blood of the freshwater turtle *Pseudemys scripta*. These changes in β_{CO_2} with P_{CO_2} arise as a result of reversible $[HCO_3^-]$ formation and proton

buffering by hemoglobin, phosphates, and plasma proteins (Piiper 1982). Changes in β_{CO_2} with increases or decreases in blood P_{CO_2} can be expected to affect CO_2 convective

transport and conductance (Piiper 1982), and therefore the respiratory exchange ratio, of *L. kempfi*.

The skeletal muscle myoglobin content ($3.1 \text{ mg} \cdot \text{g}^{-1}$ of tissue) of Kemp's ridley turtle was comparable to the value reported for the loggerhead turtle ($2.9 \text{ mg} \cdot \text{g}^{-1}$ of tissue; Lutz and Bentley 1985) and somewhat less than that reported for the leatherback turtle ($4.9 \text{ mg} \cdot \text{g}^{-1}$ of tissue; Lutcavage et al. 1990). The myoglobin concentration is substantially higher in penguins ($81 \text{ mg} \cdot \text{g}^{-1}$ of tissue; Butler and Jones 1983) and marine mammals ($72 \text{ mg} \cdot \text{g}^{-1}$ of tissue; O'Brien et al. 1992). O'Brien et al. (1992) reported that the myoglobin content is increased in species which have a high metabolic rate, physical activity fatigue resistance, and prolonged diving capacity. The blood and tissue respiratory and acid-base properties of the Kemp's ridley turtle are consistent with those of species that utilize lung O_2 stores during aerobic diving and suggest that this shallow-water coastal species is poorly adapted for prolonged deep diving.

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